

IN THE CLAIMS

Claims 1-67 (Cancelled).

68. (Withdrawn): An isolated nucleic acid molecule comprising a regulatory sequence operably linked to a nucleic acid sequence that encodes an engineered ribonucleic acid (RNA) agent, wherein the agent comprises

(i) a first stem portion comprising a sequence of at least 18 nucleotides that is complementary to a sequence of a messenger RNA (mRNA) of a target gene;

(ii) a second stem portion comprising a sequence of at least 18 nucleotides that is sufficiently complementary to the first stem portion to hybridize with the first stem portion to form a duplex stem; and

(iii) a loop portion that connects the two stem portions.

69. (withdrawn): The nucleic acid molecule of claim 68, wherein the first stem portion is fully complementary to the mRNA sequence.

70. (withdrawn): The nucleic acid molecule of claim 68, wherein the second stem portion is fully so complementary to the first stem portion.

71. (withdrawn): The nucleic acid molecule of claim 68, wherein the first stem portion is located at a 5' end of the RNA agent.

72. (withdrawn): The nucleic acid molecule of claim 68, wherein the first stem portion is located at a 3' end of the RNA agent.

73. (withdrawn): The nucleic acid molecule of claim 68, wherein the loop portion comprises at least 4 nucleotides.

74. (withdrawn): The nucleic acid molecule of claim 68, wherein the loop portion comprises at least 7 nucleotides.

75. (withdrawn): The nucleic acid molecule of claim 68, wherein the loop portion comprises 11 nucleotides.

76. (withdrawn): The nucleic acid molecule of claim 68, wherein the sequence of the mRNA is located from 100 to 300 nucleotides 3' of the start of translation of the mRNA.

77. (withdrawn): The nucleic acid molecule of claim 68, wherein the sequence of the mRNA is located in a 5' untranslated region (UTR) or a 3' UTR of the mRNA.

78. (withdrawn): The nucleic acid molecule of claim 68, wherein the first and second stem portions each comprise about 18 to about 30 nucleotides.

79. (withdrawn): The nucleic acid molecule of claim 68, wherein the first and second stem portions each comprise about 22 to about 28 nucleotides.

80. (withdrawn): The nucleic acid molecule of claim 68, wherein the first and second stem portions each comprise the same number of nucleotides.

81. (withdrawn): The nucleic acid molecule of claim 68, wherein one of the first and second stem portions comprises 1 to 4 more nucleotides than the other stem portion.

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82. (withdrawn): The nucleic acid molecule of claim 68, wherein the regulatory sequence comprises a Pol III or Pol II promoter.
83. (withdrawn): The nucleic acid molecule of claim 68, wherein the regulatory sequence is constitutive or inducible.
84. (withdrawn): A vector comprising the nucleic acid molecule of claim 68.
85. (withdrawn): The vector of claim 84, wherein the vector is a plasmid or a viral vector.
86. (withdrawn): The vector of claim 85, wherein the viral vector is a retroviral vector.
87. (withdrawn): A host cell containing the nucleic acid molecule of claim 68.
88. (withdrawn): The host cell of claim 87, wherein the cell is a mammalian cell.
89. (withdrawn): A transgene comprising the nucleic acid of claim 68.
90. (withdrawn): An engineered RNA agent comprising
- i) a first stem portion comprising a sequence of at least 18 nucleotides that is complementary to a sequence of a messenger RNA (mRNA) of a target gene; ii)a second stem portion comprising a sequence of at least 18 nucleotides that is sufficiently complementary to the first stem portion to hybridize with the first stem portion to form a duplex stem; and
 - iii) a loop portion that connects the two stem portions.

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91. (withdrawn): The agent of claim 90, wherein the first stem portion is fully complementary to the mRNA sequence.
92. (withdrawn): The agent of claim 90, wherein the second stem portion is fully complementary to the first stem portion.
93. (withdrawn): The agent of claim 90, wherein the first stem portion is located at a 5' end of the RNA agent.
94. (withdrawn): The agent of claim 90, wherein the first stem portion is located at a 3' end of the RNA agent.
95. (withdrawn): The agent of claim 90, wherein the loop portion comprises at least 4 nucleotides.
96. (withdrawn): The agent of claim 90, wherein the loop portion comprises at least 7 nucleotides.
97. (withdrawn): The agent of claim 90, wherein the loop portion comprises 11 nucleotides.
98. (withdrawn): The agent of claim 90, wherein the sequence of the mRNA is located in a 5' untranslated region (UTR) or a 3' UTR of the mRNA.
99. (withdrawn): The agent of claim 90, wherein the first and second stem portions each comprise about 18 to about 30 nucleotides.

100. (withdrawn): The agent of claim 90, wherein the first and second stem portions each comprise about 22 to about 28 nucleotides.

101. (withdrawn): The agent of claim 90, wherein the first and second stem portions each comprise the same number of nucleotides.

102. (withdrawn): The agent of claim 90, wherein one of the first and second stem portions comprises 1 to 4 more nucleotides than the other stem portion.

103. (withdrawn): The agent of claim 90, wherein the target gene is a human gene.

104. (withdrawn): The agent of claim 90, wherein the target gene is a mutant human gene.

105. (withdrawn): The agent of claim 90, wherein the target gene is a viral gene.

106. (withdrawn): A method of inducing ribonucleic acid interference (RNAi) of a target gene in a cell, the method comprising

- i) obtaining a host cell of claim 87;
- ii) culturing the cell; and
- iii) enabling the cell to express the RNA agent to form a small interfering ribonucleic acid (siRNA) within the cell, thereby inducing RNAi of the target gene in the cell.

107. (currently amended) A multitarget partially double-stranded RNA molecules comprising two or more different double stranded RNA sequences that are substantially homologous and

complementary to two or more sequences of at least one target mammalian gene or mammalian pathogen gene.

108. (previously presented) The multitarget partially double-stranded RNA molecule of claim 107, wherein said two or more different double stranded RNA sequences are substantially homologous and complementary to two or more sequences of more than one target gene.

109. (previously presented) The multitarget partially double-stranded RNA molecule of claim 107, wherein at least 11 to 30 nucleotides of said multitarget partially double-stranded RNA molecule are involved in each different double-stranded sequence.

110. (previously presented) The multitarget partially double-stranded RNA molecule of claim 107, wherein each different double stranded RNA sequence comprises at least one segment of 30 contiguous nucleotides with a homology of at least 50% to a similar 30 nucleotide region of the target sequence, wherein said segment of 30 contiguous nucleotides is also non-homologous to any naturally occurring and essential polynucleotide sequence.

111. (previously presented) The multitarget partially double-stranded RNA molecule of claim 107, wherein said multitarget partially double-stranded RNA molecule is between about 100 and 10,000 polynucleotides in length.

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112. (previously presented) The multitarget partially double-stranded RNA molecule of claim 107, wherein said multitarget partially double-stranded RNA molecule is at least about 200 nucleotides in length.

113. (previously presented) The multitarget partially double-stranded RNA molecule of claim 107, wherein one or more of said different double stranded RNA sequences comprises a sense polynucleotide and an antisense polynucleotide separated by a non-base-paired polynucleotide sequence.

114. (previously presented) The multitarget partially double-stranded RNA molecule of claim 113, wherein said sense and antisense polynucleotides form a hairpin.

115. (previously presented) The multitarget partially double-stranded RNA molecule of claim 107, wherein said two or more different double stranded RNA sequences are separated by cleavage sequences.

116. (previously presented) The multitarget partially double-stranded RNA molecule of claim 115, wherein said cleavage sequences are autocatalytic sequences or splice sites.

117. (previously presented) The multitarget partially double-stranded RNA molecule of claim 107, wherein said at least one target gene is from a single target pathogen.

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118. (previously amended) The multitarget partially double stranded RNA molecule of claim 108, wherein said more than one target genes are from more than one target pathogens.

119. (previously presented) The multitarget partially double-stranded RNA molecule of claim 117, wherein said target pathogen is a virus.

120. (previously presented) The multitarget partially double-stranded RNA molecule of claim 119, wherein said virus is selected from the group consisting of HBV, HIV, HSV, CMV, HPV, HTLV and EBV.

121. (previously presented) The multitarget partially double-stranded RNA molecule of claim 118, wherein said more than one target pathogens are viruses.

122. (previously presented) The multitarget partially double-stranded RNA molecule of claim 121, wherein said more than one viruses are selected from the group consisting of HBV, HIV, HSV, CMV, HPV, HTLV and EBV.

123. (previously presented) The multitarget partially double-stranded RNA molecule of claim 107, wherein said at least one target gene is associated with a disease or disorder in a mammal.

124. (previously presented) The multitarget partially double-stranded RNA molecule of claim 123, wherein said at least one target gene is a cancer-associated gene.

125. (previously presented) The multitarget partially double-stranded RNA molecule of claim 108, wherein said more than one target genes are associated with a disease or disorder in a mammal.

126. (previously presented) The multitarget partially double-stranded RNA molecule of claim 125, wherein said more than one target genes are cancer-associated genes.

127. (previously presented) The multitarget partially double-stranded RNA molecule of claim 107, wherein said two or more sequences of said at least one target gene are selected from the group consisting of transcribed sequences, non-transcribed sequences, coding sequences, non-coding sequences, exon-containing sequences, regulatory sequences and promoter sequences.

128. (previously presented) The multitarget partially double-stranded RNA molecule of claim 107, wherein said multitarget partially double-stranded RNA molecule lacks a poly-adenylation signal.

129. (previously presented) A composition comprising the multitarget partially double-stranded RNA molecule of claim 107.

130. (previously presented) The composition of claim 129 further comprising an agent which facilitates polynucleotide uptake by a cell.

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131. (previously presented) A DNA molecule encoding the multitarget partially double-stranded RNA molecule of claim 107.
132. (previously presented) An expression vector encoding the multitarget partially double-stranded RNA molecule of claim 107.
133. (previously presented) The expression vector of claim 132, wherein said multitarget partially double-stranded RNA molecule is expressed using a promoter selected from the group consisting of a mitochondrial promoter, a RNA pol I promoter, a RNA pol II promoter, a RNA pol III promoter, a viral promoter, a bacterial promoter and a bacteriophage promoter.
134. (previously presented) The expression vector of claim 133, wherein said multitarget partially double-stranded RNA molecule is expressed using a RNA pol III promoter.
135. (previously presented) The expression vector of claim 132, wherein said vector is a plasmid, phage or recombinant virus.
136. (previously presented) The expression vector of claim 132, wherein said encoded multitarget partially double-stranded RNA molecule lacks a poly-adenylation signal.
137. (currently amended) An expression vector for reducing or inhibiting the function of at least one target gene in a mammalian cell, wherein said expression vector encodes two or more

different double stranded sequences that are homologous and complementary to two or more sequences of said at least one target gene.

138. (previously presented) The expression vector of claim 137 wherein said two or more different double stranded RNA sequences are homologous and complementary to two or more target sequences of more than one target gene.

139. (currently amended) The expression vector of claim 137, wherein the vector encodes two or more different RNA molecules that are at least about 100 nucleotides in length, each double stranded RNA sequence comprises comprising at least 11 to 30 nucleotides involved in the double-stranded sequence.

140. (previously presented) The expression vector of claim 137, wherein each different double-stranded RNA sequence contains at least one segment of 30 contiguous nucleotides with a homology of at least 50% to a similar 30 nucleotide region of the target sequence, wherein said segment of 30 contiguous nucleotides is also non-homologous to any naturally occurring and essential polynucleotide sequence in said cell.

141. (previously presented) The expression vector of claim 137, wherein said vector encodes a multitar get partially double-stranded RNA molecule comprising two or more different double stranded RNA sequences.

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142. (previously presented) The expression vector of claim 141, wherein said multitarget partially double-stranded RNA molecule is between about 100 and 10,000 polynucleotides in length.

143. (previously presented) The expression vector of claim 141, wherein said multitarget partially double-stranded RNA molecule is at least about 200 nucleotides in length.

144. (previously presented) The expression vector of claim 137, wherein one or more of said different double stranded RNA sequences comprises a sense polynucleotide and an antisense polynucleotide separated by a non-base-paired polynucleotide sequence.

145. (previously presented) The expression vector of claim 144, wherein said sense and antisense polynucleotides form a hairpin.

146. (previously presented) The expression vector of claim 141, wherein said two or more different double stranded RNA sequences are separated by cleavage sequences.

147. (previously presented) The expression vector of claim 146, wherein said cleavage sequences are autocatalytic sequences or splice sites.

148. (previously presented) The expression vector of claim 137, wherein said two or more different double stranded RNA sequences are expressed using a promoter selected from the

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group consisting of a mitochondrial promoter, a RNA pol I promoter, a RNA pol II promoter, a RNA pol III promoter, a viral promoter, a bacterial promoter and a bacteriophage promoter.

149. (previously presented) The expression vector of claim 148, wherein said one or more promoters are RNA pol III promoters.

150. (previously presented) The expression vector of claim 137, wherein said two or more different double stranded RNA sequences are expressed using two or more promoters.

151. (previously presented) The expression vector of claim 144, wherein said two or more different double stranded RNA sequences are expressed using two or more promoters.

152. (previously presented) The expression vector of claim 150, wherein said two or more promoters are selected from the group consisting of a mitochondrial promoter, a RNA pol I promoter, a RNA pol II promoter, a RNA pol III promoter, a viral promoter, a bacterial promoter and a bacteriophage promoter.

153. (previously presented) The expression vector of claim 152, wherein said two or more promoters are RNA pol III promoters.

154. (previously presented) The expression vector of claim 151, wherein said two or more promoters are selected from the group consisting of a mitochondrial promoter, a RNA pol I

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promoter, a RNA pol II promoter, a RNA pol III promoter, a viral promoter, a bacterial promoter and a bacteriophage promoter.

155. (previously presented) The expression vector of claim 154, wherein said two or more promoters are RNA pol III promoters.

156. (previously presented) The expression vector of claim 137, wherein said vector is a plasmid, phage or recombinant virus.

157. (previously presented) The expression vector of claim 137, wherein said at least one target gene is from a single target pathogen.

158. (previously presented) The expression vector of claim 138, wherein said more than one target genes are from more than one target pathogens.

159. (previously presented) The expression vector of claim 157, wherein said target pathogen is a virus.

160. (previously presented) The expression vector of claim 159, wherein said virus is selected from the group consisting of HBV, HIV, HSV, CMV, HPV, HTLV and EBV.

161. (previously presented) The expression vector of claim 158, wherein said more than one target pathogens are viruses.

162. (previously presented) The expression vector of claim 161, wherein said more than one viruses selected from the group consisting of HBV, HIV, HSV, CMV, HPV, HTLV and EBV.

163. (previously presented) The expression vector of claim 137, wherein said at least one target gene is associated with a disease or disorder in a mammal.

164. (previously presented) The expression vector of claim 163, wherein said at least one target gene is a cancer-associated gene.

165. (previously presented) The expression vector of claim 138, wherein said more than one target genes are associated with a disease or disorder in a mammal.

166. (previously presented) The expression vector of claim 165, wherein said more than one target genes are cancer-associated genes.

167. (previously presented) The expression vector of claim 137, wherein said two or more sequences of said at least one target gene are selected from the group consisting of transcribed sequences, non-transcribed sequences, coding sequences, non-coding sequences, exon-containing sequences, regulatory sequences and promoter sequences.

168. (withdrawn) The expression vector of claim 137, wherein said two or more different double stranded RNA sequences lack a polyadenylation signal.

169. (withdrawn) A method of making a composition comprising two or more different double stranded RNA molecules comprising expressing the expression vector of claim 137 in a cell.
170. (withdrawn) The composition comprising two or more different double stranded RNA molecules produced by the method of claim 169.
171. (previously presented) A multitarget partially double-stranded RNA molecule encoded by the expression vector of claim 137.
172. (previously presented) A composition comprising the expression vector of claim 137.
173. (previously presented) The composition of claim 172 further comprising an agent which facilitates polynucleotide uptake by a cell.
174. (new) The expression vector of claim 137, wherein the vector encodes two or more different RNA molecules that are less than about 750 nucleotides in length, each comprising at least 11 to 30 nucleotides involved in the double-stranded sequence.